Human Organic Anion Transporters and Human Organic Cation Transporters Mediate Renal Transport of Prostaglandins

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ABSTRACT

Prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) and prostaglandin F\textsubscript{2\alpha} (PGF\textsubscript{2\alpha}) have been used for the induction of labor and the termination of pregnancy. Renal excretion is shown to be an important pathway for the elimination of PGE\textsubscript{2} and PGF\textsubscript{2\alpha}. The purpose of this study was to elucidate the molecular mechanism of renal PGE\textsubscript{2} and PGF\textsubscript{2\alpha} transport using cells stably expressing human organic anion transporter (hOAT) 1, hOAT2, hOAT3, and hOAT4, and human organic cation transporter (hOCT) 1 and hOCT2. A time- and dose-dependent increase in PGE\textsubscript{2} and PGF\textsubscript{2\alpha} depletion (Siragy and Carey, 1997). Therapeutically, since enhanced in pathophysiological settings including sodium Levine, 1973; Terragno et al., 1976), and this conversion is way for the elimination of PGE\textsubscript{2} and PGF\textsubscript{2\alpha} secretion (Irish, 1979). Since PGE\textsubscript{2} and PGF\textsubscript{2\alpha} possess anionic moieties (Fig. 1, A and B), it is suggested that urinary excretion of PGE\textsubscript{2} and PGF\textsubscript{2\alpha} is mediated by the organic anion transport system. However, little is known about the molecular mechanism of the tubular secretion of PGE\textsubscript{2} and PGF\textsubscript{2\alpha}.

The secretory or excretory function of the renal tubules is a two-step process consisting of active, energy-dependent basolateral uptake followed by passive apical secretions (Irish, 1979). Studies with perfused kidney tubules indicated that active vectorial PG transport across the epithelium is a two-step process consisting of active, energy-dependent basolateral uptake followed by passive apical secretions (Irish, 1979). Since PGE\textsubscript{2} and PGF\textsubscript{2\alpha} possess anionic moieties (Fig. 1, A and B), it is suggested that urinary excretion of PGE\textsubscript{2} and PGF\textsubscript{2\alpha} is mediated by the organic anion transport system. However, little is known about the molecular mechanism of the tubular secretion of PGE\textsubscript{2} and PGF\textsubscript{2\alpha}.

The secretion of numerous organic anions and cations, including endogenous metabolites, drugs, and xenobiotics, is an important physiological function of the renal proximal tubule. The process of secreting organic anions and cations through the proximal tubule cells is achieved via unidirectional transcellular transport, involving the uptake of organic anions and cations into the cells from the blood across the basolateral membrane, followed by extrusion across the brush-border membrane into the proximal tubule fluid (Pritchard and Miller, 1993). Recently, cDNAs encoding organic anion transporter (OAT) family have been successively cloned including OAT1 (Sekine et al., 1997; Sweet et al., 1997; Hosoyamada et al., 1999; Lu et al., 1999), OAT2 (Simonsen et al., 1994; Sekine et al., 1998), OAT3 (Kusuhara et al., 1999; Cha et al., 2001) and OAT4 (Cha et al., 2000). The

Prostaglandins (PGs) play various physiological and pathophysiological roles. Among them, PGE\textsubscript{2} and PGF\textsubscript{2\alpha} are the predominant cyclooxygenase metabolites of arachidonic acid in the kidney (Breyer and Badr, 1996). PGE\textsubscript{2} and PGF\textsubscript{2\alpha} play an important role in the tubular reabsorption of salt and water as well as in the control of renal vascular resistance and the maintenance of glomerular hemodynamics. PGE\textsubscript{2} is known to be converted enzymatically to PGF\textsubscript{2\alpha} (Leslie and Levine, 1973; Terragno et al., 1976), and this conversion is enhanced in pathophysiological settings including sodium depletion (Siragy and Carey, 1997). Therapeutically, since both PGE\textsubscript{2} and PGF\textsubscript{2\alpha} have potent oxytocic actions, the synthetic preparations of these compounds have been used to terminate pregnancy and to facilitate labor (Foegh and Ramwell, 2001). In addition, PGE\textsubscript{2} has also been used for the treatment of gastric and duodenal ulcers, especially those induced by nonsteroidal anti-inflammatory drugs (Altman DF, 2000). Pharmacokinetically, it has been reported that 63% of PGE\textsubscript{2} and 55.7% of PGF\textsubscript{2\alpha} administered were excreted into the urine in rats (Nishihori and Matsuoka, 1971; Nishihori et al., 1973). In addition, studies with perfused kidney tubules indicated that active vectorial PG transport across the epithelium is a two-step process consisting of active, energy-dependent basolateral uptake followed by passive apical secretions (Irish, 1979). Since PGE\textsubscript{2} and PGF\textsubscript{2\alpha} possess anionic moieties (Fig. 1, A and B), it is suggested that urinary excretion of PGE\textsubscript{2} and PGF\textsubscript{2\alpha} is mediated by the organic anion transport system. Although little is known about the molecular mechanism of the tubular secretion of PGE\textsubscript{2} and PGF\textsubscript{2\alpha}.

The secretion of numerous organic anions and cations, including endogenous metabolites, drugs, and xenobiotics, is an important physiological function of the renal proximal tubule. The process of secreting organic anions and cations through the proximal tubule cells is achieved via unidirectional transcellular transport, involving the uptake of organic anions and cations into the cells from the blood across the basolateral membrane, followed by extrusion across the brush-border membrane into the proximal tubule fluid (Pritchard and Miller, 1993). Recently, cDNAs encoding organic anion transporter (OAT) family have been successively cloned including OAT1 (Sekine et al., 1997; Sweet et al., 1997; Hosoyamada et al., 1999; Lu et al., 1999), OAT2 (Simonsen et al., 1994; Sekine et al., 1998), OAT3 (Kusuhara et al., 1999; Cha et al., 2001) and OAT4 (Cha et al., 2000). The

**ABBREVIATIONS:** PG, prostaglandin; OAT, organic anion transporter; OCT, organic cation transporter; hOAT, human OAT; hOCT, human OCT; PAH, para-aminobiphenyl acid; ES, estrone sulfate; D-PBS, Dulbecco’s modified phosphate-buffered saline; TEA, tetraethylammonium.
organic cation transporters (OCTs) isolated so far are OCT1 (Grundemann et al., 1994; Zhang et al., 1998), OCT2 (Okuda et al., 1996; Busch et al., 1998). Among these clones, human OAT1 (hOAT1), hOAT2, hOAT3, and hOCT2 were shown to be localized to the basolateral side of the proximal tubule (Gorouole et al., 1997; Hosoyamada et al., 1999; Cha et al., 2001; Pietig et al., 2001; unpublished observation), whereas hOAT4 was localized to the apical side of the proximal tubule (Babu et al., 2002).

The purpose of this study was to elucidate the molecular mechanism of renal PGE2 and PGF2α transport. For this purpose, we established the proximal tubule cells stably expressing hOAT1, hOAT2, hOAT3, hOAT4, hOCT1, and hOCT2.

**Experimental Procedures**

**Materials.** [3H]PGE2 (7,159.5 GBq/mmol) and [3H]PGF2α (7,943.2 GBq/mmol) were purchased from Amersham Biosciences UK, Ltd. (Buckinghamshire, UK). 14C-Labeled para-aminobenzoic acid (PAH) (18648 GBq/mmol), [3H]estrone sulfate (ES) (1961 GBq/mmol) and [3H]PAE (3,962 GBq/mmol) were purchased from Amersham Biosciences UK, Ltd. (Buckinghamshire, UK). [3H]PGE2 and [3H]PGF2α were obtained from Sigma-Aldrich (St. Louis, MO). Other materials used included fetal bovine serum, trypsin, and genetin from Invitrogen (Carlsbad, CA), recombinant epidermal growth factor from Nakunaga (Hiroshima, Japan), insulin from Shimiizu (Shizuoka, Japan), RTC 80-7 culture medium from Iwaki Co. (Tokyo, Japan) and TIU-50 from Promega (Madison, WI).

**Cell Culture and Establishment of the Second Portion of Proximal Tubule (S2) Cells Stably Expressing hOAT2, hOAT4, hOCT1, and hOCT2.** S2 cells were established by culturing the microdissected S2 segment derived from transgenic mice harboring the temperature-sensitive simian virus 40 large T-antigen gene (Hosoyamada et al., 1996). During this establishment period, the functions of OATs and OCTs may have been lost. However, among the various cell lines stably expressing rat OAT3 that we have established, i.e., S2 cells, the terminal proximal tubule (S3) cells, Chinese hamster ovary cells and LLC-PK1 cells, we found that S2 cells alone exhibited phorbol 12-myristate 13-acetate-induced down-regulation of organic anion uptake, which is recognized in the intact proximal tubule (Takeda et al., 2000b). Thus, we suggest that S2 cells possess the essential machinery for the regulation of the OAT system and are the most suitable host for analyzing the OAT system. The establishment and characterization of S2 hOAT1 and S2 hOAT3 were reported previously (Takeda et al., 2000a). The full-length cDNA of hOAT2 was isolated by screening human kidney cDNA library using rat OAT2 cDNA as a probe (Babu et al., 1998). The full-length cDNA of hOCT1 was obtained by reverse-transcription and polymerase chain reaction of cDNA using primers spanning the coding region of the published sequence of hOCT1 (Gorouole et al., 1997). The full-length cDNA of hOCT2 was isolated by screening human kidney cDNA library using rat OCT2 cDNA (Okuda et al., 1996) as a probe. The full-length cDNAs of hOAT2, hOAT4 (Cha et al., 2000), hOCT1, and hOCT2 were subcloned into pCDNA3.1 (Invitrogen), a mammalian expression vector. S2 hOAT2, S2 hOAT4, S2 hOCT1, and S2 hOCT2 were obtained by transfecting S2 cells with pCDNA3.1-hOAT2, pCDNA3.1-hOAT4, pCDNA3.1-hOCT1, and pCDNA3.1-hOCT2 coupled with pSV2neo, a neomycin resistance gene using TFX-50 according to the manufacturer’s instructions. S2 cells transfected with pCDNA3.1 lacking an insert and pSV2neo were designated as S2 pCDNA3.1 (mock), and used as control. These cells were grown in a humidified incubator at 33°C and under 5% CO2 using RTC 80-7 medium containing 5% fetal bovine serum, 10 mg/ml transferrin, 0.08 U/ml insulin, 10 ng/ml recombinant epidermal growth factor, and 400 mg/ml geneticin. The cells were subcultured in a medium containing 0.05% trypsin-EDTA solution (containing 137 mM NaCl, 5.4 mM KCl, 5.5 mM glucose, 4 mM NaHCO3, 0.5 mM EDTA, and 5 mM Hepes; pH 7.2) and used for 25 to 35 passages. Clonal cells were isolated using a cloning cylinder and screened by determining the optimal substrate for each transporter, i.e., [3H]PAH for hOAT1 (Hosoyamada et al., 1999), [3H]PGE2 for hOAT2 (unpublished observation), [3H]ES for hOAT3 and hOAT4 (Cha et al., 2000, 2001) and [3H]TEA for hOCT1 and hOCT2 (Okuda et al., 1996; Zhang et al., 1998).

**Uptake Experiments.** Uptake experiments were performed as previously described (Takeda et al., 1999, 2000a,b, 2002). The S2 cells were seeded in 24-well tissue culture plates at a cell density of 1× 105 cells/well. After the cells were cultured for 2 days, the cells were washed three times with Dulbecco’s modified phosphate-buffered saline (D-PBS) solution (containing 137 mM NaCl, 3 mM KCl, 8 mM NaHCO3, 1 mM KH2PO4, 1 mM CaCl2, and 0.5 MgCl2; pH 7.4), and then preincubated in the same solution in a water bath at 37°C for 10 min. The cells were then incubated in a solution containing either [3H]PGE2 or [3H]PGF2α at various concentrations as indicated in each experiment at 37°C for 1 min. The uptake was stopped by the addition of ice-cold D-PBS, and the cells were washed three times with the same solution. The cells in each well were lysed with 0.5 ml of 0.1 N sodium hydroxide and 2.5 ml of aquasol-2, and radioactivity was measured using a β-scintillation counter (LSC-3100; Aloka, Tokyo, Japan).

**Inhibition Study.** To evaluate the inhibitory effects of PGE2 and PGF2α on organic anion uptake in S2 hOAT1, S2 hOAT2, S2 hOAT3, and S2 hOCT1, and S2 hOCT2, the cells were incubated in a solution containing either 5 μM [3H]PAH for 2 min (for hOAT1), 50 nM [3H]PGE2, for 1 min (for hOAT2), 50 nM [3H]ES for 2 min (for hOAT3 and hOAT4), or 5 μM [3H]TEA for 5 min (for hOCT1 and hOCT2) in the absence or presence of PGE2 and PGF2α at 37°C as described above. PGE2 and PGF2α were dissolved in dimethyl sulfoxide and diluted with the
incubation medium. The final concentration of dimethyl sulfoxide in the incubation medium was adjusted to less than 1%.

**Statistical Analysis.** Data are expressed as means ± S.E. Statistical differences were determined using the Student’s unpaired t test. Differences were considered significant at $P < 0.05$.

**Results**

**PGE$_2$ and PGF$_{2\alpha}$ Uptake Mediated by hOATs and hOCTs.** We have already observed that $S_2$ hOAT2 exhibited a time- and dose-dependent uptake of PGF$_{2\alpha}$ with a $K_m$ value of 425 nM (unpublished observation). We further elucidated the time-dependent uptake of PGE$_2$ and PGF$_{2\alpha}$ in $S_2$ cells stably expressing hOATs and hOCTs. As shown in Fig. 2, $S_2$ hOAT1 (A), $S_2$ hOAT2 (B), $S_2$ hOAT3 (C), $S_2$ hOAT4 (D), $S_2$ hOCT1 (E), and $S_2$ hOCT2 (F) exhibited higher PGE$_2$ uptake than mock. Similarly, as shown in Fig. 3, $S_2$ hOAT1 (A), $S_2$ hOAT3 (B), $S_2$ hOAT4 (C), $S_2$ hOCT1 (D), and $S_2$ hOCT2 (E) exhibited significantly higher PGF$_{2\alpha}$ uptake than mock. The kinetics of PGE$_2$ and PGF$_{2\alpha}$ uptake were examined to evaluate the pharmacological characteristics of hOATs and hOCTs on the uptake of PGE$_2$ and PGF$_{2\alpha}$. Figure 4 shows the Eadie-Hofstee plots of the concentration dependence of PGE$_2$ uptake by $S_2$ hOAT1 (A), $S_2$ hOAT2 (B), $S_2$ hOAT3 (C), $S_2$ hOAT4 (D), $S_2$ hOCT1 (E), and $S_2$ hOCT2 (F) after subtraction of uptake by mock. The estimated $K_m$ values of PGE$_2$ uptake by hOAT1, hOAT2, hOAT3, hOAT4, hOCT1, and hOCT2 are listed in Table 1. On the other hand, Fig. 5 shows the Eadie-Hofstee plots of concentration dependence of PGF$_{2\alpha}$ uptake by $S_2$ hOAT1 (A), $S_2$ hOAT3 (B), $S_2$ hOAT4 (C), $S_2$ hOCT1 (D), and $S_2$ hOCT2 (E) after subtraction of uptake by mock. The estimated $K_m$ values of PGF$_{2\alpha}$ uptake by hOAT1, hOAT2, hOAT3, hOAT4, hOCT1, and hOCT2 are also listed in Table 1. These results suggest that PGE$_2$ and PGF$_{2\alpha}$ transport is mediated by hOAT1, hOAT2, hOAT3, hOAT4, hOCT1, and hOCT2.

**Inhibitory Effects of PGE$_2$ and PGF$_{2\alpha}$ on Organic Anion and Organic Cation Uptake.** We examined the inhibitory effects of PGE$_2$ and PGF$_{2\alpha}$ on organic anion uptake mediated by hOAT1, hOAT2, hOAT3, and hOAT4, and organic cation uptake mediated by hOCT1 and hOCT2. Table 2 shows that PGE$_2$ significantly inhibited organic anion uptake mediated by hOAT1, hOAT2, hOAT3, and hOAT4 ($n = 4$, *$P < 0.001$ versus control), and organic cation uptake mediated by hOCT1 and hOCT2 ($n = 4$, *$P < 0.001$ and **$P < 0.01$ versus control). Similarly, PGF$_{2\alpha}$ exerted a significant inhibitory effect on hOAT1-, hOAT2-, hOAT3-, and hOAT4-
mediated organic anion uptake ($n = 4$, $*P < 0.001$ versus control), and hOCT1- and hOCT2-mediated organic cation uptake (hOCT1: $n = 4$, $*P < 0.001$ versus control; hOCT2: $n = 4$, $**P < 0.01$ versus control).

**Discussion**

hOAT1 and hOAT3 were shown to mediate the transport of nonsteroidal anti-inflammatory drugs, antitumor drugs, histamine H2 receptor antagonist, PGIs, diuretics, angiotensin-converting enzyme inhibitors, and β-lactam antibiotics (Hosoyamada et al., 1999; Cha et al., 2001). Some differences
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in characteristics exist between hOAT1 and hOAT3, such as substrate specificity and localization: hOAT1 at the basolateral side of the S3 segment of the proximal tubule (Hosoyamada et al., 1999) versus hOAT3 at the first, second, and third segments (S1, S2, and S3) of the proximal tubule (Cha et al., 2001). In addition, hOAT1, but not hOAT3, exhibits transport properties as an exchanger (Hosoyamada et al., 1999; Cha et al., 2001). HOAT2, also shown to be localized to the basolateral side of the proximal tubule, mediates the transport of organic anions including salicylate and PGF2α (unpublished observation). HOAT4 also mediates the apical transport of various anionic drugs; however, this transporter exhibits relatively narrow substrate recognition compared with hOAT1 and hOAT3 (Cha et al., 2000; Babu et al., 2002).

HOCT1 was shown to be mainly localized to the liver and to mediate polyspecific pH independent transport of organic cations, whereas organic cation transport by hOCT2 was pH independent, electrogenic, and polyspecific. (Gorboulev et al., 1997; Busch et al., 1998; Zhang et al., 1998).

At present, limited information is available concerning renal tubular excretion of PGE2 and PGF2α. PG transporter-mediated transport of PGE2 and the inhibitory effect of PGE2 on rat-OAT1-mediated organic anion transport were reported (Kanai et al., 1995; Sekine et al., 1997). In this regard, the current results revealed that multiple pathways exist concerning the transport of PGs in the basolateral side of the proximal tubule, i.e., hOAT1, hOAT2, hOAT3, and hOCT2, whereas we could not exclude the possibility that transporters other than those analyzed in this study are also involved in PGE2 and PGF2α transport.

In addition to basolateral transporters, the characterization of the interaction between PGE2 or PGF2α and apical OATs is also important. In this regard, we found that hOAT4, an apical transporter of renal tubules, mediates the uptake of PGE2 as well as and PGF2α. In addition, we already observed that hOAT4 mediates the efflux of ES (Babu et al., 2002). Thus, it is possible that hOAT4 mediates the bidirectional transport of PGs on the apical side of the proximal tubule. On the other hand, we could not find the functional characteristics of hOAT4 as an exchanger (Cha et al., 2000). Instead, when the amount of PG reabsorbed from the tubular lumen together with that taken up from the basolateral side reaches a certain threshold that activates the hOAT4-mediated efflux, PG reabsorption from the tubular lumen may contribute to the secretion of PG into the tubular lumen. At present, the role of other apical transporters mediating organic anion transport remains unknown, including OAT-K1 (Saito et al., 1996), OAT-K2 (Masuda et al., 1999), organic anion-transporting peptide 1 (Jacquemin et al., 1994), multiple drug resistance protein 2 (Leier et al., 2000), and human type I sodium-dependent inorganic phosphate transporter (Uchino et al., 2000), and further study should be performed to elucidate this.

As shown in Fig. 1, PGE2 and PGF2α possess anionic moieties. This is consistent with the current results that hOAT1, hOAT2, hOAT3, and hOAT4 mediate the transport of PGE2 and PGF2α. In contrast, since both PGE2 and PGF2α possess no cationic moieties, hOCT1- and hOCT2-mediated PGE2 and PGF2α uptakes were unexpected. Various substrates were shown to be transported via the OAT as well as the OCT system, and are called bisubstrates (Ullrich et al., 1993a, b). In this regard, PGE2 and PGF2α could also be regarded as bisubstrates. Recently, we also found that acyclovir and ganciclovir are transported by hOAT1 as well as hOCT1 (Takeda et al., 2002). In addition, hOATs including hOAT1, hOAT2, and hOAT3 and hOCT2 are colocatalized in the basolateral side of the proximal tubule (Gorboulev et al., 1997; Hosoyamada et al., 1999; Cha et al., 2001; Pietig et al., 2001; unpublished observation), and it is possible to design a model in which PGE2 and PGF2α are transported via OAT and OCT at the same time. Further studies should be performed to identify the mechanism underlying the involvement of OCTs in the transport of PGE2 and PGF2α.

In conclusion, the current results suggest that hOATs as well hOCTs are responsible for the transport of PGE2 and PGF2α in the basolateral side as well as the apical side of the renal tubule. The current results provide important information for safer and more efficient clinical use of PGE2 and PGF2α. Particular attention must be taken when these PGE2 and PGF2α are concomitantly used with other drugs that share common transporters with these PGs for tubular excretion. Otherwise, concomitant administration of PGE2 or PGF2α with other drugs may induce the increase in plasma concentrations of these PGs or other drugs, resulting in adverse drug reactions.

References


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